Name of Organization: Michigan State University & MDEQ

Type of Organization: College or University

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Project Title: Pharmaceuticals & Resistant Bacteria in MI Surface Waters

Project Category: Emerging Issues

Rank by Organization (if applicable): 0

Total Funding Requested ($): 356,440 **Project Duration:** 2 Years

Abstract:

The focus of the proposed research is twofold. First, novel instrumental techniques will be used to analyze surface waters for pharmaceutical compounds including antibiotics of human and animal origin. Selection criteria for targeted drugs will be based on usage statistics, potential for endocrine disruption, and persistence in the environment. Target drugs used in humans include amoxicillin, tetracycline, Fasomax and related agents (osteoporosis treatment), ethynyl estradiol,Tamoxifen and related products (breast cancer treatments), and equilin, clofibrate, and related lipid lowering agents. The target veterinary drugs used in cattle, poultry, swine, and aquaculture will include Ceftiofur, tetracycline, sulfonamides, gentamycin and fluoroquinolone antibiotics. Efforts will be made to screen for other persistent drugs as techniques and equipment allow. Secondly, surface water samples will be screened for the presence of antibiotic-resistant bacteria of animal origin that are human and wildlife pathogens. The drug-resistant strains of bacteria monitored in surface waters are expected to be serotypes of Salmonella, E.coli and Campylobacter. These three genera in the environment are primarily of animal orgin but are both human and animal pathogens. Virulent and non-virulent strains of E.coli will be isolated for further characterization. The virulence factors that will be tested include attaching and effacing genes, Shiga toxins and heat stable enterotoxin genes.

Geographic Areas Affected by the Project

States:

- ☐ Illinois
- ☐ Indiana
- ☒ Michigan
- ☐ Minnesota
- ☐ New York
- ☐ Pennsylvania
- ☐ Wisconsin
- ☐ Ohio

Lakes:

- ☐ Superior
- ☐ Huron
- ☐ Michigan
- ☐ Erie
- ☐ Ontario
- ☒ All Lakes

Geographic Initiatives:

☐ Greater Chicago ☐ NE Ohio ☐ NW Indiana ☒ SE Michigan ☐ Lake St. Clair

Primary Affected Area of Concern:

Other Affected Areas of Concern:

For Habitat Projects Only:

Primary Affected Biodiversity Investment Area:

Other Affected Biodiversity Investment Areas:

Problem Statement:

The occurrence of pharmaceutical drugs in surface waters raises public health concerns for potential genotoxic and endocrine disruptor effects in animals and pathogenic outbreaks in humans and animals. Pharmaceutical drugs are often designed to pass through biological membranes and persist in the target tissues of animals or humans for prophylactic effects. As these compounds are metabolized and excreted, they enter the environment either as unchanged substances or in different chemical configurations, including mixtures of metabolites that may consist of parent compounds conjugated with an inactivating substituent (e.g. glutathione or sulfate). Conjugated compounds may be de-conjugated during wastewater treatment or in surface waters. Hospital wastewater has been an important point source for drugs used in humans. Active pharmaceutical agents also may reach surface waters from nonpoint pathways as large quantities of drugs are used as growth promoters, therapeutics, and anti-parasitic agents in intensive swine, dairy cattle, beef cattle, poultry, and aquaculture production.

Another important public health concern related to the use of pharmaceuticals is the occurrence of antibiotic-resistant strains of pathogenic bacteria in the environment. Strains of bacteria possessing virulent attributes that have the capacity to cause diarrhea and systemic diseases in humans are potentially a serious threat to humankind. Continuous, chronic exposure to low-levels of antibiotics destroys sensitive bacteria and selects for resistant traits in remaining bacterial populations. This results in an increase in the population of antibiotic-resistant bacteria. Since bacteria in wastewater may be continually exposed to antibiotics, there is potential for the presence of a variety of drug-resistant pathogenic bacteria in surface waters. Typical examples include the drug-resistant strains of E. coli that result from the treatment of cattle with antibiotics, and penicillin-resistant Staphylococcus pneumoniae, a bacteria that causes ear infections, pneumonia, and meningitis, which have been reported in environmental samples. This suggests the necessity for surveillance of antibiotic-resistant bacteria in waters.

Proposed Work Outcome:

The Surface Water Quality Division (SWQD) and MSU will work cooperatively to develop the sampling strategy and SWQD will provide logistical support for sample collection. Selection criteria for water sampling locations will be based upon the existence of possible sources (residential, agricultural, commercial, hospital). We propose to select 5 swine farms, 5 large dairy farms, 5 poultry farms, 5 beef feed lots and 5 urban WWTPs in Southeast Michigan watersheds. Samples will be collected 6 times (every two months) in a year at each of the selected sampling sites. Information regarding the historical use of antimicrobials (therapeutic and subtherapeutic) will be collected for each farm. For wastewater treatment facilities, information on the type and extent of treatment and the volume treated per day will be collected. A reference site which is away from the source of effluent discharge, in the Great Lakes, will also be included.

For the analysis of pharmaceuticals, samples will be filtered and extracted at the sampling location to avoid degradation of compounds during transport. MSU has advanced analytical tools and techniques to perform the study. High performace liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LS-MS) techniques will be used to identify the target pharmaceutical drugs.

For bacteriological analysis, sterilized vials will be used for sampling. Samples will be placed on ice in coolers, transported to the laboratory and immediately set up for culture. Bacteriologic examinations will be performed in accordance with standard diagnostic procedures for each bacterial species described in the American Society for Microbiology Manual of Clinical Microbiology (1995) and Clinical Veterinary Microbiology (1994). Bacteria will be identified using extractions of DNA, PCR techniques, and conventional identification schemes. Susceptibility of bacteria to 11 antibiotics will be performed using standard disk diffusion techniques. Results will be interpreted as described in the National Committee for Clinical Laboratory Standards (NCCLS) M31-T document. Also, the number and types of drug-resistant strains of Salmonella, E. coli and Campylobacter will be examined. Select virulent strains will be stored to allow for detection of detailed resistances by determining Minimum Inhibitory Concentrations (MIC) and mechanisms by which the strains develop resistance.

This project is designed to determine the presence and identification of pharmaceuticals and antibiotic-resistant strains of pathogenic bacteria in ponds, water storage facilities, wastewater treatment plant effluents, and surface waters. The results of this study will provide information on the quality of Michigan waters in regard to the types of potential endocrine disrupting chemicals and so called human health microorganisms that are found. The study will also provide information on the environmental persistency of pharmaceuticals and the efficacy of wastewater treatment processes in removing pharmaceuticals. Information on the types and number of antibiotic-resistant bacteria will provide information to develop preventive measures. Also, the relationship between the presence of drugs and drug-resistant strains will be evaluated. Information from this project will be shared and if warranted, further investigation will continue to determine the impact that pharmaceuticals and antibiotic-resistant pathogenic bacteria have on the environment and human health.

Project Milestones:	Dates:
Project initiation	06/2000
Project sampling	08/2000
Method development	12/2000
Water sampling	03/2001
Sample analysis	03/2001
Data summary	11/2001
Data analysis	01/2002
Report preparation	03/2002

☐ Project Addresses Environmental Justice

If So, Description of How:

☐ Project Addresses Education/Outreach

If So, Description of How:

This project will be part of graduate training for university students. Also, the Michigan Department of Environmental Quality, Surface Water Quality Division will be collaborating with water sampling and dissemination of information. An inter-net website, conference presentations and peer reviewed publications will also be used to disseminate results and new information.

Project Budget:

	Federal Share Requested ($)	Applicant's Share ($)
Personnel:	143,500	39,000
Fringe:	29,910	35,000
Travel:	12,710	1,000
Equipment:	26,000	20,000
Supplies:	114,800	0
Contracts:	0	0
Construction:	0	0
Other:	29,520	0
Total Direct Costs:	356,440	95,000
Indirect Costs:	0	0
Total:	356,440	95,000
Projected Income:	0	0

Funding by Other Organizations (Names, Amounts, Description of Commitments):

Applications are being prepared for submittal to the National Institute of Health (NIH) and the US Department of Agriculture (USDA). Applications have already been sent to the Michigan Great Lakes Protection Fund and to the US Food and Drug Administration (FDA). The FDA was supportive of this proposal and willing to provide funds as long as another granting source would initiate funding. We have begun to approach pharmaceutical companies, such as Pharmacia and Upjohn in Kalamazoo, Michigan. We will use the GLNPO funds as both seed funds and matching funds for industry and foundation support. We propose to continue to expand the locations, compounds and bacteria that we investigate.

Description of Collaboration/Community Based Support:

The Surface Water Quality Division of the Michigan Department of Environmental Quality and the National Food Safety and Toxicology Center of Michigan State University will be collaborating on this project. This assembled team is unique in its ability to address the chemical and biological components as well as providing technical equipment and expertise to conduct the proposed research project.

Dr. Giesy is well known for his work on endocrine disrupters in the Great Lakes region. He serves on the National Academy of Science Panel on Endocrine Disrupters and is a World Health Organization (WHO) advisor on endocrine disrupters as a member of the EU/USA task force on joint research.

Dr. Holland is a veterinarian and works extensively with antibiotic uses, resistance problems and infectious diseases of farm animals.

Dr. Kannan is an environmental chemist with experience in the analysis of a wide variety of environmental contaminants in various environmental media. Dr. Kannan has experience in handling HRGC-HRMS, HPLC and HPLC-MS.

Dr. Briggs is a toxicologist working on human risk assessment and surface water quality issues for the state of Michigan.